Biphasic Scaffolds for Bone Repair: Nanofiber-Permeated Pore Network for Enhanced Cell Population

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Statement of Purpose: Sintered composite microsphere matrices have shown progress toward fulfilling the goal of autograft replacement, but studies have suggested that cellular migration is often limited to the periphery in static culture. As collagen represents the largest organic component of functional bone, fibrous networks of the physical scale of collagen ECM in bone may increase cellular retention and migration, leading to improved bone repair. While a fibrous structure alone would have limited clinical utility due to no load bearing potential, we propose to increase cell migration throughout a mechanically stable microsphere matrix using a secondary, nanofibrous phase within its pore structure. We hypothesize that a nanofiber mesh can be synthesized within the pore structure and potentially increase cell migration and residence throughout the scaffold.

Methods: Microsphere matrices were fabricated according to reported procedure[1]. Briefly, to synthesize composite microspheres, poly(L-lactide) (PLLA) was dissolved in methylene chloride in a 1:5 w/v polymer/solvent ratio. The PLLA solution was added to a stirring solution of 1% polyvinyl alcohol. The suspension was mixed for 24 hours to allow the methylene chloride to evaporate and microspheres to form. After mixing, formed microspheres were rinsed, isolated, and placed in stainless steel molds heated for 90 minutes to bond adjacent microspheres. Microsphere scaffolds were then submerged in a three separate concentrations (0.25%, 1%, and 2%) of PLLA in dimethylformamide (DMF) and submerged in liquid nitrogen to allow thermally induced phase separation (TIPS) to occur[2]. After freezing, the scaffolds were immersed in DI water changed three times over 24 hours to ensure total extraction of the DMF. After solvent extraction, the nanofiber/microsphere hybrid scaffolds were lyophilized to remove residual water and stored under vacuum. Mechanical integrity of hybrid scaffolds was measured using an Instron Uniaxial Testing Machine (Instron, Norwood, MA). Compressive modulus and the maximum compressive strength of control and hybrid scaffolds were determined from load-displacement data using Merlin data analysis software (Instron). For cell culture, MC3T3 cells were seeded onto the scaffolds at a density of $2x10^5$ cells per scaffold and allowed to adhere for 20 minutes and then cultured in growth medium for 7 days, bisected, then stained using a live/dead viability assay and imaged using confocal microscopy.

Results: The results from the 1% TIPS hybrid scaffold are shown in Figure 1. Notably, the fiber diameter visible at 1000x closely mimics the collagen fiber diameter seen in human ECM (50-500 nm). Also evident are a series of interconnected pores in the TIPS nanofiber network on the order of 10 μm, the size of a human osteoblast. Mechanical data are shown in figure 2 and demonstrate compressive modulus within the range of trabecular bone [3], with no significant decrease in modulus with the addition of the nanofiber phase. Results of the 7-day viability study for the 0.25% hybrid scaffold are shown in figure 3. Viable cells appear to occupy the interior of the scaffold including some cells that appear to adhere solely to ECM-mimetic fibers.

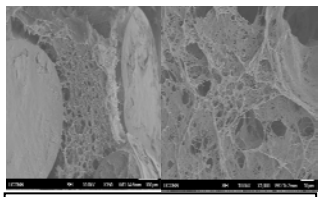

Figure 1. (left) 150x mag. SEM of 1% hybrid scaffold interior;(right) 1000x mag. showing nanofibrous structure

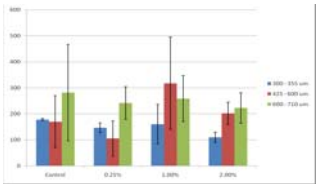

Figure 2. Mechanical data. Y-axis shows compressive modulus (MPa)

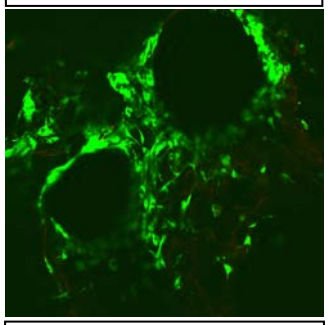

Figure 3. Confocal microscopy showing viable cells (green) in the interior of the hybrid scaffold

Conclusions: This study evaluated the possibility of incorporating a secondary, ECM-mimetic phase into the pore structure of a load bearing, sintered microsphere scaffold. Results indicate that an ECM-mimetic fibrous network was evident as assessed by scanning electron micrographs. Importantly, PLLA TIPS fibrous networks share a similar size to natural ECM networks on the order to 50-500 μm and have pore spaces large enough to facilitate human osteoblast migration throughout the pore structure. Networks of this size have been implicated in affecting growth, adhesion, proliferation, and differentiation of cells involved in bone repair[4]. An ECM-mimetic network with pores large enough for osteoblasts to migrate through might allow increased migration and subsequent bone remodeling by osteoblasts or osteoblast precursors.

References: [1] Wang QQ. Biomed Mater. 6(5): 055009, 2011 [2] Wei G. Biomaterials. 25(19):4749-57, 2004 [3] Parfitt AM, JBMR 2(6):595-6107. [4] Borden M. Biomaterials. 23(2):551-9, 2002